a reprint from

Developments in Food Preservatives–1

Edited by

R. H. TILBURY

Published by

APPLIED SCIENCE PUBLISHERS LTD

Ripple Road, Barking, Essex, England

addition, sequestering agents, other preservatives and reducing agents. New methods in experimental design have enabled investigators to examine these variables.

A special problem with pH-dependent food preservatives exists because, as with water content, it is difficult to determine where pH boundaries exist. Measurements of pH, water content, or salt concentration should be considered on a non-homogeneous basis. Bacterial contamination in whole pieces or portions of food exists in layers, and similar profiles exist also with pH, water content, salt content, and even preservative concentration. Bryan[1] refers to this type of physical boundary as 'interfaces' when the boundaries are different components of a food, such as a cake and its filling, meat emulsion and the protein skin, and crust and crumb of bread, which allows differences in concentration of the essential elements for food preservation (pH, water, salt, preservative). In a two-component food such as a filled cupcake, the cake portion can be above pH 4·5 and the filling below pH 4·5 with differences also in the water activity (A_w). When does such a food become a hazard on the basis of pH and A_w? Bakery products with cream fillings, regardless of the pH of the filling, should be classified as hazardous foods requiring continual refrigeration. Because of the interface and the effects of diffusion or migration in terms of contamination and preservation action, many food products which heretofore have been considered non-hazardous should be re-examined for bacteriological safety.

In an excellent review on the mechanism of anti-microbial action of food preservatives, Oka[2] concluded that, although much knowledge has been obtained on the inhibition of metabolic processes in microbial cells by chemicals used as food preservatives, the factors that determine the anti-microbial effect remain undetermined. For effective activity, the chemical component must be transferred from the medium into the cell, and preservatives have been classified according to the manner of this transfer process. The effectiveness of preservatives in the absorption group is determined by the amount of preservative absorbed. Another group acts by oxidation of coenzymes within the cell. Both mechanisms are affected by cell permeability. Therefore, the lipophilicity of the compound and the surface activity of the cell membrane are important factors in the determination of food preservative efficiency. Thus, the destruction of cells is dependent on the interaction of the compound with specific sites on the membrane, rupturing the membrane and exposure of the cell components to the environment.[2]

Some research areas remain. What is the mechanism for inhibitory action? Do food preservatives lower heat resistance? Are preservatives absorbed and pass through the cell membrane, or do they react with the membrane?

The use of food preservatives in the 1980s should have much the same status as anti-biotics had in the 1960s when the emphasis and main objectives in research were not screening and identification of new anti-biotics, but rather the development of new, more efficient systems and conditions for the use of existing anti-biotics.

This chapter presents a review on the status of present preservatives in new systems and combinations; discussion on past research in such areas as anti-biotics for food preservation, radiation and special packaging; and the re-evaluation of these methods in the light of recent findings on mechanisms and effectiveness of traditional food preservatives. The contribution of fatty acids, anti-oxidants and plant extracts in food preservation, and new approaches being investigated to integrate packaging with the anti-microbial requirements for perishable foods, are also discussed.

NITRITE—NEW DEVELOPMENTS

Nitrite has had a long history as meat preservative; however, in recent years its use has been under attack because of the production of nitrosamines from the combination of nitrite with secondary amines. Knowledge of the mode of anti-bacterial action of nitrite will enable researchers to design substitutes that will mimic nitrite action against bacteria. The demonstration that iron (Fe^{2+} and Fe^{3+}) antagonises the anti-botulinal action of nitrite may indicate how nitrite behaves against micro-organisms.

Combining nitrite with reducing agents enhanced the activity of nitrite against *Clostridium botulinum* in meat slurries and in perishable canned cured meats.[3–6] Ascorbate, isoascorbate and cysteine were effective in enhancing the anti-bacterial action of nitrite. Thioglycollate had some activity, whereas sodium formaldehyde sulphoxylate, sodium formaldehyde bisulphite, or sodium sulphide possessed no nitrite-enhancing activity. Thus, reducing activity *per se* did not lead to enhancement of anti-botulinal effects of nitrite. None of the compounds that enhanced nitrite activity had any effect against *C. botulinum* in the absence of nitrite.

When isoascorbate and nitrite were present in perishable canned cured pork there was considerable delay in the outgrowth of *C. botulinum* compared to that in cans with nitrite alone. The combination of isoascorbate and nitrite added to perishable canned cured pork hearts gave

an unexpected result—there was no inhibition of *C. botulinum* outgrowth.[5] However, when the isoascorbate and nitrite combination was supplemented with EDTA, *C. botulinum* outgrowth was prevented in the canned pork hearts. Pork hearts contain approximately four times the iron that pork ham contains,[7] and it is suggested that the amount of isoascorbate used in the formulation was not enough to bind all of the iron present in canned cured pork hearts unless EDTA was added also.

Since EDTA is a known sequestrant of divalent metallic ions, Tompkin *et al.*[5] postulated that ascorbate, isoascorbate and cysteine, like EDTA, enhance nitrite anti-bacterial action by chelating an essential metal required by *C. botulinum* for outgrowth. However, Morris *et al.*[8] observed that ascorbate (and presumably isoascorbate) was an excellent chelator of Cu^{2+} but bound Fe^{3+} poorly. Therefore, ascorbate enhancement of nitrite anti-botulinal activity may be due to factors other than iron binding.

In later work, Tompkin *et al.*[7,9] showed that addition of iron (Fe^{2+} or Fe^{3+}) to canned perishable cured beef or pork containing nitrite allowed the outgrowth of *C. botulinum*. The addition of Mg^{2+}, Mn^{2+}, or Zn^{2+} did not have a similar effect on nitrite. Thus, a supply of available iron completely abolishes the anti-botulinal activity of nitrite. Unfortunately, the authors did not attempt to reverse the effect of iron by the addition of EDTA.

Tompkin *et al.*[7] postulated that nitric oxide (from nitrite) combined with ferrodoxin (or a similar iron-containing compound) in germinated *C. botulinum* spores and prevented energy metabolism needed for outgrowth. An excess of available iron in the meat product combines with nitrite and prevents its anti-bacterial action. A search for suitable sequestrants for use in meat products could very well result in greatly decreased nitrite levels.

STARTER CULTURE INHIBITORS

It has been known for many years that there are natural inhibitory systems in raw milk. Under certain circumstances when raw milk is added to a food, growth of many bacteria are inhibited. Antibiotic-like and other inhibitory substances produced by various members of the family *Lactobacteriaceae* and extracted from culture media have been identified as diplococcin, nisin and several other anti-biotics of lesser activity.[10] Diplococcin is known to inhibit most streptococci and other gram-positive cocci. Nisin has a broad spectrum of activity including several streptococci groups. Other inhibitory substances found in dairy substrates inoculated with starter cultures include acids (primarily lactic and acetic acids), CO_2, hydrogen peroxide, polypeptides and certain volatile fatty acids.[11] The lactoperoxidase/thiocyanate/H_2O_2 system found in milk is also bactericidal. The hydrogen peroxide is produced by lactic acid bacteria, and the lactoperoxidase and thiocyanate are found in milk.[12]

It is an acceptable practice in the cheese industry to add lactic acid culture to milk when received under conditions that produce only a slight increase in titratable acidity and/or decrease in pH. The object is to inhibit bacterial growth during the holding time in the plant and to condition the milk protein at the same time for coagulation by the clotting enzyme treatment which follows. In cottage cheese production, cream innoculated with *Streptococcus cremoris*, *S. lactis* and *S. diacetilactis*,[13] can be added to cream the curd. This processing step prevents slime formation and increases shelf-life of the final product. Butter cultures have been used for many years to produce desired flavour and to inhibit the growth of various bacteria that cause flavour defects in butter. The culture organisms compete with spoilage organisms for nutrients and oxygen.

There is limited use of starter cultures in other fermented foods, or fermentations are often carried out sometimes by the normal lactic flora found in the foods. Manufacturers of fermented foods other than fermented milk products seem reluctant to change their traditional processes.

In the production of dry and semi-dry fermented sausages, fewer than half of the processors use lactic acid starter cultures. Few of the fermented sausages sold in local supermarkets list starter culture on the label.[14] The reluctance of meat processors to use starter cultures should be overcome because the advantages of using starter cultures are manifold: (1) large numbers of bacteria of proven lactic acid producing capacity are added; (2) more rapid acid production decreases the chance of undesirable organisms taking over the fermentation; and (3) processing time is decreased, since it is not necessary to allow for the growth of the natural lactic flora to numbers sufficient to initiate fermentation.

In the absence of starter culture, growth of *Staphylococcus aureus* in a thuringer-type sausage was not affected; when fermentation was initiated by a lactic acid starter culture containing *Pediococcus cerevisiae* and/or *Lactobacillus plantarum*, growth of *S. aureus* was prevented.[15] Also, in the absence of starter culture, measurable amounts of staphylococcal enterotoxin A were detected in European dry sausages.[16]

In summer-style sausage, Christiansen *et al.*[17] demonstrated that the mixture of *L. plantarum* and *P. cerevisiae* prevented toxin formation by

C. botulinum in 23 of 25 sausages; addition of 50 ppm nitrite with the starter culture prevented toxin formation completely. Omission of glucose led to large numbers of toxic sausages. It is necessary that the processor add sufficient fermentable carbohydrate to obtain inhibitory concentrations of lactic acid.

Smith *et al.*[18,19] demonstrated that *Salmonella dublin* and *S. typhimurium* were killed more consistently during lebanon bologna and pepperoni processing when starter cultures containing a mixture of *P. cerevisiae* and *L. plantarum* were used to initiate the fermentation. In natural flora-fermented sausages, a heat treatment was necessary to ensure destruction of *Salmonellae*. Thus, sausage fermentations initiated by addition of lactic acid starter cultures were more effective than those initiated with natural flora in preventing growth and toxin formation by food-poisoning bacteria.

Products such as fermented vegetables (cucumbers and sauerkraut) purchased in local supermarkets do not have starter cultures listed on the labels. Although the advertising literature indicates that starter cultures are available for fermented vegetables, they are probably not in common use. Lactic acid starter cultures would be advantageous to the vegetable fermentation industry by decreasing spoilage and the growth of food-poisoning micro-organisms.

Lactic acid bacteria can be added to a variety of meat products and pasteurised liquid whole egg to prevent both the growth of spoilage organisms and food-borne pathogens. Raccach and Baker[20] found that lactic acid bacteria protect pasteurised liquid whole egg from spoilage (the spoilage organisms could be present as a result of post-processing contamination or underpasteurisation), but the starter was not effective in preventing the growth of *S. typhimurium*. However, in cooked, mechanically deboned poultry meat, the same starter culture inhibited the growth of *S. typhimurium*.[21] Thus, the potential effects of lactic acid bacteria cannot be predicted from one food to another.

Bryan[22] reported that staphylococcal food poisoning is found frequently in hams and ham products. Starter cultures may be useful in preventing growth of staphylococci in ham products. *Streptococcus diacetilactis* present in temperature-abused (25°C) ham sandwich spread killed *Staphylococcus aureus*.[23] Bartholomew and Blumer[24] showed that *P. cerevisiae* repressed the growth of normal ham flora. While they did not study the effect of starter culture on *S. aureus*, the authors suggested that addition of lactic acid bacteria might be useful in preventing growth and toxin formation by food-borne pathogens in hams.

The shelf-life of ground beef could be extended by the addition of a variety of lactic acid bacteria.[23,25] Although not directly related to preservation by lactic acid bacteria, the addition of 2% glucose increased the shelf-life of ground beef by several days.[26] There was no change in the normal flora; the spoilage organisms preferentially used the added sugar, produced a lower pH, and repressed their own growth. When all of the available glucose was utilised, the micro-organisms began to metabolise the nitrogenous compounds of meat, leading to an increased pH, off-odours and surface slime. Addition of at least 2% glucose increased the low-temperature shelf-life of ground beef from 5 days to 8–10 days.

Studies by Riemann and his co-workers[27] indicate that semi-preserved meats containing radiation-killed *P. cerevisiae* would not be a food-poisoning hazard. Under conditions of temperature abuse, the killed pediococci utilise fermentable carbohydrates (normally added to semi-preserved meats) to produce acid which would prevent the growth of food-borne pathogens. Under refrigeration, the radiation-killed pediococci would be inactive. This technique could be applied to other foods.

In fermented products, lactic acid is the chief agent in the prevention of spoilage and growth of food pathogens; the reason for the protective action of lactic acid bacteria in unfermented products is unclear. Inhibitory substances such as anti-biotics, H_2O_2 and unidentified compounds have been found in lactic acid bacterial culture fluids.[10,11,28] Recent work on the purification of fermentation liquor obtained from the growth of lactic acid bacteria in culture broth indicates that active anti-bacterial substances are present.[29,30] Further work is needed to identify the compounds and to establish whether it would be possible to add them to foods as preservatives.

FATTY ACIDS AND DETERGENTS

The antiseptic and disinfectant properties of soap are considered to be from residual alkalinity. However, the anti-microbial action is still apparent even at low concentrations of soap in the range 5–50 ppm. At these concentrations, the ionisation of the fatty acid is not important. This has interesting implications in food preservation because fatty acids can be used with wide tolerances in foods.

The anti-microbial effects of small amounts of long-chain fatty acids have been documented by Nieman;[31] gram-positive bacteria are generally more susceptible than gram-negative organisms. A common feature of detergents (which include long-chain fatty acids), phenols, quaternary

ammonium compounds and polypeptide anti-biotics is their ability to bind to the cell membrane and cause its disruption as the semi-permeable barrier between cell and environment.[32]

Long-chain fatty acids are not bactericidal to the tubercle bacillus at neutral pH but become so under acid conditions. A virulent strain of *Mycobacterium tuberculosis* normally resistant to 0·1 N HCl (pH 1·0) was rapidly killed at the low pH when traces of fatty acids (C_{12}, C_{14}, C_{16}, $C_{18:1}$) were present.[33] Kondo and Kanai[34] tested fatty acids against various species of mycobacteria; long-chain fatty acids (C_{14}, $C_{18:1}$, $C_{19:2}$) were active against all test strains.

With *M. bovis*, the unsaturated long-chain fatty acids inhibited membrane-bound enzyme activity (acid phosphatase and tetrazolium reductase) as well as growth.[34] Oleic acid had only slight mycobactericidal activity at pH 7 but had very strong activity at pH 5·6. Addition of the basic protein protamine to oleic acid at acid pH led to loss of mycobactericidal activity.[34] Further work by Kondo and Kanai[35] indicated that bactericidal effects of long-chain fatty acids was of non-specific nature and was due to the detergent-like action on the cytoplasmic membrane. The difference in sensitivity between gram-positive and gram-negative bacteria was shown to be due to the ability of the former to adsorb long-chain fatty acids.

The growth of gram-positive organisms belonging to the genera *Bacillus*, *Streptococcus*, *Staphylococcus*. *Micrococcus* and *Clostridium* was inhibited by sodium laurate or sodium linolenate (0·5 mM or less at pH 7·4 in bacterial media). Bacilli were inhibited by lower concentrations of the long-chain fatty acid than were the other genera. Gram-negative cells were not affected.[36] Lauric acid was the most effective saturated fatty acid tested but was not as effective as C_{18} unsaturated fatty acids. Calcium and magnesium ions, cholesterol and ergocalciferol reversed the fatty acid inhibition of growth. Kabara *et al.*[37,38] studying gram-positive cocci (streptococci, micrococci and pneumococci), found that lauric acid was the most active saturated long-chain fatty acid. The monoglyceride, 1-monolaurin, was more active in preventing bacterial growth than was the free acid; 1,3-dilaurin or the triglyceride had no effect.

Utilising *B. subtilis*, Sheu and Freese[39] showed that the concentration of fatty acid required to inhibit growth increased with decreasing molecular weight (pH 7 in bacterial media); 0·5 mM $C_{18:2}$ gave as much inhibition as did 100 mM C_4. Freese *et al.*[40] reviewed the anti-microbial properties of lipophilic food additives. Most lipophilic food preservatives prevent bacterial growth by inhibiting the transport of substrate molecules into cells. Saturated long-chain fatty acids uncouple transport of substrate and oxidative phosphorylation from the electron transport system and inhibit cellular uptake of amino acids, organic acids and phosphate through the membrane. Unsaturated long-chain fatty acids also appear to inhibit the electron transport system of the cell.

At pH 6·0, lauric acid was effective against *B. megaterium* and *M. lysodeikticus*; with increasing pH, a higher concentration of the fatty acid was necessary to show growth inhibition (0·03 mM at pH 6 as compared to 0·3 mM at pH 8). The anti-bacterial effect of linoleic acid decreased with increasing pH but to a lesser degree than that of lauric acid.[41] The uptake of both lauric and linoleic acids by *B. megaterium* and *M. lysodeikticus* was governed by pH also. The amount of fatty acid uptake decreased as the pH increased and reflected the decrease in bactericidal activity. Although *Pseudomonas phaseolicola* was resistant to the inhibitory action of long-chain fatty acids, it did adsorb these fatty acids. Protoplasts of *B. megaterium* had a greater uptake of the long-chain fatty acids than had whole cells; thus, removal of the cell wall allows greater exposure of the cell membrane to the fatty acids. Galbraith and Miller[42] showed that addition of bovine serum albumin antagonised the lytic action of lauric and linoleic acids on protoplasts of *B. megaterium*. The added protein may have absorbed the fatty acids, removing them from the cellular environment, and prevented cell lysis.

Galbraith and Miller[42] also studied the degree of protoplast lysis by measurement of leakage or release of purines, pyrimidines, proteins and peptides. The molar concentration of capric acid (C_5) necessary to produce lysis was 10 times greater than that of lauric acid or linoleic acid.

Long-chain fatty acids stimulated oxygen uptake by *B. megaterium* and *M. lysodeikticus* at bactericidal concentrations but inhibited oxygen uptake at high concentrations.[43] Protoplasts were more susceptible than whole cells to inhibition. Oxygen uptake by whole cells of *P. phaseolicola* was not inhibited by lauric or linoleic acids but spheroplasts were susceptible to the action of the fatty acids.[43] Long-chain fatty acids inhibited the uptake of glutamic acid and lysine by *B. megaterium* and *M. lysodeikticus*; the inhibiting effect was reversed by Ca^{2+}. The uptake of lysine was prevented by lauric and linoleic acids in *C. welchii*. High levels of fatty acids were necessary to inhibit lysine or glutamate uptake in *P. phaseolicola* and *Escherichia coli* but spheroplasts of the gram-negative cells were just as sensitive as were gram-positive whole cells. Long-chain fatty acids (C_{12}, C_{14}, C_{18}, $C_{18:2}$) caused leakage of glutamate from cells of *B. megaterium* preloaded with radioactive glutamate. Thus, the work of Galbraith and Miller indicates that long-chain fatty acids act on the

cytoplasmic membrane (site of O_2 uptake and transport of amino acids) probably involving the uncoupling of energy systems.

Sheu and Freese[44] examined the lack of inhibition of most gram-negative bacteria by long-chain fatty acids. Growth, amino acid transport and O_2 consumption in *E. coli* and *Salmonella typhimurium* were inhibited by short-chain fatty acids (C_{2-6}) but not by medium- or long-chain fatty acids (C_{10-18}). The resistance is not correlated with the ability of the cells to metabolise fatty acids but is due to the nature of the lipopolysaccharide (LPS) layer which surrounds gram-negative bacteria. The LPS layer prevents accumulation of long-chain fatty acids on the inner cell membrane (site of amino acid transport). Inhibition of the growth of an *E. coli* mutant defective in the LPS layer occurred with levels of decanoate that were not effective with normal *E. coli*. However, the LPS defective mutant was not inhibited by oleate. Addition of EDTA to the gram-negative organisms removed part of the LPS layer, and the cells then became sensitive to long-chain fatty acids (C_{10} and $C_{18:2}$). Fay and Farias[45] also showed that spheroplasts of *E. coli* were sensitive to methyldecanoate at levels that had no effect on whole cells. Sheu and Freese[44] suggested that the addition of EDTA along with lipophilic food preservatives would increase the effectiveness of those inhibitors against gram-negative bacteria.

Kato and Arima[46] found that lauric acid inhibited growth of *E. coli* more effectively than did other fatty acids (C_{10}–$C_{18:3}$) tested. The authors synthesised the water-soluble sucrose mono-ester of lauric acid, which was inhibitory at 100 μg/ml (1 mg/ml lauric acid showed partial inhibition only). However, the sucrose ester was only bacteriostatic, and growth of *E. coli* occurred after a long lag. Kabara[47] found that sucrose laurate was no more active than lauric acid against members of *Streptococcus* group D or *Staphylococcus epidermis*.

Relatively low concentrations of long-chain fatty acids are inhibitory to gram-positive bacteria in bacterial media. Unfortunately, the long-chain fatty acids are not effective against gram-negative bacteria. The inhibitory effect of the long-chain fatty acids is more marked at lower pH, and the inhibitory action is reversed by additions of proteins or by ions such as Ca^{2+}.[42,43] Gram-negative cells are more affected by short-chain fatty acids.[44] It might appear feasible to formulate foods with short-chain fatty acids to lower the pH; addition of long-chain fatty acids along with the short-chain fatty acids would inhibit both gram-positive and gram-negative bacteria. However, the effect of such additives on the flavour and physical characteristics of food must be considered. The precise composition and the physical and chemical form or condition of the fatty acid additive can be well defined and controlled by sophisticated analytical methods. Simple dispersion of a fatty acid in a food cannot be effective for food preservation purposes. It may be critical, for example, that the fatty acid exist as unimolecular films so that they form boundaries or cover large surface areas. Also, the fatty acid may exist in some complex form with proteins, carbohydrates, or some other forms of lipid material.

ANTI-OXIDANTS AS INHIBITORS

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are frequently added to foods because of their anti-oxidant properties; however, these hindered phenols are similar to other phenols in possessing anti-microbial activity.

Chang and Branen[48] reported that the growth and aflatoxin production of *Aspergillus parasiticus* were prevented by 250 ppm BHA in growth medium. Fung *et al.*[49] found that growth of toxin formation were inhibited in six strains of *A. flavus* by BHA (0·005–0·02 g/petri dish). BHT at similar concentrations had no effect.

Staphyloccus aureus growing in nutrient broth was quite sensitive to BHA: 150–200 ppm was bactericidal. The bactericidal level of BHA for enteropathogenic *E. coli* was 400 ppm, but such levels delayed the growth of *Salmonella typhimurium* only slightly.[48]

In a nutrient medium, 50 ppm BHA rapidly killed *Vibrio parahaemolyticus*. However, in a sterile homogenate of blue crabmeat, 400 ppm was necessary to exert a bactericidal effect. Robach *et al.*[50] suggested that the decrease in the anti-bacterial effect of BHA in crabmeat was due to partial inactivation of the BHA by oxidised lipids. The anti-bacterial effect of BHA in foods, therefore, may depend on the content and type of lipids present.

Klindworth *et al.*[51] tested BHA against *Clostridium perfringens* in fluid thioglycollate medium and found that 200 ppm inhibited the growth of the organism. BHA was stable to autoclaving and reacted synergistically with nitrite, sorbic acid and parabens. Unfortunately, the addition of lipid to the medium drastically reduced the effectiveness of BHA against *C. perfringens*.

Although BHA shows anti-microbial activity in culture media, its utility as a food preservative has not been demonstrated. The decrease in activity found when lipids are added suggests that the anti-bacterial activity and the anti-oxidant effect are linked and that BHA which reacts with lipids is no

longer available for anti-bacterial activity. Alternately, lipid inactivation of the anti-bacterial properties of BHA may be due to solubilisation of the anti-oxidant in the lipid, thus making it unavailable for action upon micro-organisms.

SEQUESTRANTS

Sequestrants (chelating agents) react with metals to form complexes that in many cases effectively bind the metal ions and remove them as reactants or catalysts in reactions that cause deterioration of the food. Many sequestrants occur naturally in foods; these include polycarboxylic acids (oxalic, succinic), hydroxycarboxylic acids (citric, malic, tartaric), polyphosphoric acid (ATP), amino acids (glycine, leucine, cysteine) and various macromolecules (porphyrins, peptides and proteins). Metal chelating agents can act alone or synergistically with other compounds.[52]

There are biological sequestrants that have been shown to have anti-bacterial activity. Micro-organisms, like other biological forms, require metallic ions (especially iron) for growth and metabolism. For example, conalbumin, an iron-binding protein found in egg-white, has inhibited the growth of various micro-organisms and such inhibition is reversed by iron.[53] Washing eggs in water containing iron will accelerate spoilage.[54] Thus, the iron-sequestering action of conalbumin protects eggs from microbial deterioration. Lactoferrin, the milk iron-binding protein, inhibits the growth of *E. coli in vitro*, and the effect is abolished by the addition of iron.[55,12] There are no reports concerning the possibility that lactoferrin protects milk or milk products from microbial spoilage.

Higher organisms can obtain iron from the foods they ingest, but bacteria must extract iron directly from the environment. Since iron is insoluble, the concentration available for bacterial growth is extremely low. Therefore, bacteria produce small organic molecules (siderochromes or siderophores) that facilitate transport of iron into the cell.[56] Bacteria also produce other chelating agents to facilitate uptake of metallic ions other than iron. Weinberg[57] suggested that the addition of microbial sequestrants (or analogues thereof) to food products may be useful in the prevention of microbial spoilage or to prevent the growth of food-borne pathogens. Feeding mice a basal diet supplemented with enterobactin (an iron-binding agent produced by a number of gram-negative species) protected them against *Salmonella typhimurium* infection.[58]

Fresh meats spoil quickly at low temperatures because of the growth of *Pseudomonas* species. Since these bacteria require iron for growth and obtain it from the meat substrate, the addition of the specific microbial sequestrant to bind iron could prevent the bacterial growth that leads to meat spoilage.

The possibilities for control or inhibition of bacterial growth in specific foods by control and manipulation of iron availability by use of synthetic or natural sequestrants remain to be studied.

CONTROLLED ATMOSPHERES

The aerobic meat spoilage *Pseudomonas* species are inhibited by use of carbon dioxide in storage and packaging. Carbon dioxide atmospheres for storage of fruits and vegetables retard respiration, ripening and the growth of bacteria. The systems used for fruits and vegetables require carbon dioxide generators and air-tight bins or containers in refrigerated storage areas. Significant progress has been made with in-package controlled atmosphere systems in which films are used with selective permeabilities of oxygen and carbon dioxide.[59] In such a system there is a gradual accumulation of carbon dioxide in the package due to respiration of the fruit or vegetable product. The ratio of carbon dioxide to oxygen is maintained to inhibit growth of anaerobic bacteria which produce off-flavours.

Benedict *et al.*,[60] utilising a mixture of sodium bicarbonate and citric acid, developed an in-package system for the generation of CO_2 in packaged meat. The gas is produced as the moisture within the package increases and effectively retards the growth of principal meat spoilage organisms. Other studies have been conducted to evaluate the effect of various gas atmospheres on microbial growth, pH and colour of fresh meat during storage.[61] Samples were stored in barrier bags which were evacuated of air and flushed with gas and sealed. Samples stored in CO_2 had significantly lower aerobic bacterial counts than N_2, O_2, or air, while anaerobic bacterial counts did not increase during 27 days of storage.

The risk in vacuum packaging in former years was in the maintenance of low oxygen atmospheres because of weaknesses in the package seal or in the package material itself. These problems have been generally eliminated by new progress in packaging technology; for example, the hypobaric transport and storage of fresh foods utilises a precisely controlled combination of low pressure, low temperature and high humidity to achieve extended shelf-life.[62] Anaerobic bacterial growth, which would not be

inhibited in vacuum packages, is also a problem. This entire area of technology of gaseous atmospheres for preservation by anti-bacterial action should be evaluated for different product classes (meat, fish, dairy and other protein-containing foods). This objective has been incidental in earlier studies because of the more urgent demands for retardation of staling, rancidity, flavour loss, and other storage effects of chemical deterioration.

INHIBITION BY REDUCED WATER ACTIVITY

Sugars and sugar alcohols are being used in special food applications to reduce water activity and thus inhibit microbial growth. It is a common practice to add salts, sugars and glycols to foods for this purpose, and such ingredients may be self-limiting because of saltiness, sweetness and bitterness. Novel approaches to manipulate the chemical and physical structure of foods and the distribution of water in the food have been studied. The outgrowth of these studies probably will be a new generation of technology and product applications that will allow storage of foods without refrigeration or thermal processing; minimal changes in flavour, colour and texture will occur in these foods compared with their conventional or traditional counterparts.

The lowered water activity is probably necessary to make water unavailable for solubilisation and transport of nutrients required for the growth of micro-organisms. One approach to accomplish this objective is to reduce the amount of immobilised water held in the tissue structure of the food by the principle of hydrostatic equilibrium or osmotic drying. The piece of vegetable or meat is placed in a high solids medium such that a hydrostatic pressure differential is developed; the water inside the food piece will tend to migrate toward the concentrated solution. Depletion of water inside the cell then destroys the nutritional balance of water-soluble nutrients for the growth of micro-organisms. At the same time, depletion of water causes concentration of salts, which will denature the protein components of micro-organisms. Other approaches are as follows.

Enzymes

Enzymes effective in the hydrolysis of plant gums and breakdown of macromolecules *in situ* to effect localised hydrostatic differences in the intracellular spaces can be added to foods. This approach will provide a shorter pathway to transfer water from areas of low solids (higher water content) to areas of higher solids content. Lower-molecular-weight species or breakdown products from complex polysaccharides and proteins are converted to simpler soluble compounds. In the lower-molecule-weight form, hydration as well as solubility will be enhanced, giving greater concentration differentials and finally more effective hydrostatic action.

Hydrogen Bonding

Hydrogen-bond breakers, such as urea or quanidinum chloride which would accelerate the dissociation of water from polar groups of protein and carbohydrates, should be studied. The hydrogen-bond breaking mechanism may help to produce unequal moisture regions, allowing hydrostatic forces to work.

Water of Hydration

Water that is chemically free is in the optimum state for chemical and bacteriological activity. Increases in the water of hydration may have larger effects on the distribution and balance of free water and create unfavourable conditions for micro-organisms.

LIQUID SMOKE

One of the difficulties in assessing the anti-bacterial properties of smoke is that heating and drying effects are also part of the smoking process. However, previous research over the years indicates that smoking imparts anti-bacterial and anti-oxidant properties to foods.

Liquid smoke preparations can be diluted and added to bacteriological media in such a way that a quantitative measure of the anti-bacterial properties of the smoke can be made. The factors of heating and drying are eliminated. Unfortunately, liquid smoke preparations are selected for their flavour qualities rather than for their anti-bacterial properties and thus have not been studied extensively from a bacteriological viewpoint. The composition of the 'smoke' flavour fraction of CharSol C-10 has been described by Fiddler *et al.*[63,64] The ether-soluble fraction that has the smoke flavour contained primarily phenols and carbonyls. The anti-bacterial potential of the fraction was not studied.

Handford and Gibbs[65] prepared smoked water concentrates by smoking water in plastic casings. The resultant smoke liquids were tested against various micro-organisms. They found that 22 of 39 members of the family *Lactobacteriaceae* were not inhibited by the smoke preparations, whereas

only 2 of 26 catalase-positive cocci were resistant. *Staphylococcus aureus* strains were strongly inhibited. Handford and Gibbs also showed that smoking tended to accelerate the shift from a predominant catalase-positive flora (micrococci) to a lactic acid flora during storage of vacuum-packed sliced bacon. Thus, the data obtained with the bacon studies were substantiated by the model system liquid smoke studies with bacterial media.

Houben[66] tested five different smoke preparations against micro-organisms growing in bacteriological media. One preparation was strongly bacteriostatic, two had moderate activity, and two were inactive. *Bacillus* species and *Microbacterium* species were quite sensitive to liquid smoke, but *E. coli* and members of the family *Lactobacteriaceae* were resistant. Two of the preparations were tested in meat suspensions by Houben[67] against *B. cereus*, *B. subtilis*, *E. coli*, *S. aureus*, *Streptococcus faecium* and one species each of *Lactobacillus*, *Microbacterium* and *Micrococcus*. CharSol EZC85 inhibited growth of *S. aureus* (at about the 1·3 % level), but the other micro-organisms were not affected by 2 % (the highest level used). Naarden IM142 inhibited the bacilli and *Microbacterium* at the 0·15 % level or less and *Lactobacillus* at 0·7 %, but did not affect the other bacteria. Houben's work indicated that anti-bacterial potential among various smoke preparations may differ greatly.

If liquid smoke preparations are selected for both flavour and anti-bacterial activity, then liquid smoke can play a role in preserving those foods that are normally smoked.

NATURAL PHENOLS AND RELATED COMPOUNDS

Over the years various researchers have conducted screening-type investigations on the anti-bacterial activity of plant extracts. In one such investigation, Powers[68] studied anthocyanin, leucoanthocyanins and phenol acids. He tested 24 compounds for their effect on respiration or reproduction of bacterial cells. When glucose was present, anthocyanin and leucoanthocyanin inhibited *E. coli*, *Salmonella typhosa*, *Aerobacter aerogenes*, *Proteus vulgaris* and several other bacterial species. In the absence of glucose, the bacterial cells metabolised these plant pigments. *Para*-hydroxybenzoic acid, gallic acid and vanillic acid inhibited *E. coli*, *A. aerogenes* and *P. vulgaris* in human urine.

Extracts prepared from certain wood species and containing phenolic neoflavanoids (and isomers of cinnamylphenols) exhibited anti-bacterial activity against a variety of gram-positive bacteria, yeasts and moulds. Jurd *et al.*[69] reported that the minimal inhibitory concentration compared favourably with several synthetic anti-microbial agents. Extracts were prepared by Smale *et al.*[70] from fresh leaves, stems and flowers of 125 plant species and varieties for measurement of anti-microbial activity, and 21 of 45 active extracts showed sufficient activity and potency to warrant further study. Bloomfield[71] studied the mechanism of action of phenolic anti-bacterial agents and concluded that these compounds disrupted active transport in the cell membrane. The phenolic compounds accelerate proton translocation across the membrane. Bloomfield obtained experimental evidence for this conclusion by studying the bacteriostatic action of Fentichlor against *S. aureus* and *E. coli*.

IRRADIATION

Extensive research and development work has been completed on the use of ionising radiation as a method for food preservation. The subject has been thoroughly reviewed by Urbain.[72] In addition, the *Proceedings of the International Symposium on Food Preservation by Irradiation* are available from the International Atomic Energy Agency.[73] The subject areas covered at this symposium were: Control of Microbial Spoilage; Chemical Changes; Toxicological Studies; Public Health and Consumer Acceptance; Economics and Energy Aspects; Irradiation Facilities.

It is generally known that low doses of ionising radiation, in the order of 10^4 roentgen equivalent physical (REP), results in a significant increase in the shelf-life of many perishable food products. At such low doses, many bacterial species that cause spoilage survive. For more effective pasteurisation, doses of 10^6 REP must be used. With fresh meat, however, this treatment allows only a moderate extension of shelf-life. Higher doses cause changes in the organoleptic properties of the food, particularly flavour and colour. Radiation treatment combined with other preservatives has been highly effective. In one such example, Wierbicki *et al.*[74] reported that it is possible to reduce the levels of nitrite and nitrates in cured meats and still have a microbiologically safe product. In irradiation-sterilised cured meats, nitrite can be reduced to levels needed only for colour and flavour. However, small amounts of nitrate must be added to prevent fading of cured meat colour. In irradiation-sterilised ham and corned beef, the total added nitrate and nitrite should be 75 ppm (1/3 to 1/2 nitrite). No nitrosamines were found in fully cooked smoked hams after radiation

treatment. In radiation-sterilised prefried bacon, a 1:1 mixture of nitrate and nitrite at 50 ppm gave a satisfactory product. After additional frying to crispness, no nitrosopyrrolidine was found.

Radiation sterilisation is probably the most underutilised method for food preservation. However, three problem areas are associated with irradiation for food preservation: (1) chemical changes which adversely affect colour, flavour and consumer acceptability of the food; (2) the toxicological status of the compounds formed by irradiation treatment; and (3) high cost for irradiation processing and marketing difficulties. Up to the present, there are no product advantages of irradiated foods that could justify higher consumer costs. A joint committee of the Food and Agricultural Organisation/International Atomic Energy Agency/World Health Organisation accepted the concept of food irradiation as a 'food process' rather than a 'food additive'. In addition, they approved several irradiated food items, including chicken, cod and redfish. Some experts are advocating the approval of classes of food because of obvious similarities in their photochemistry.

Preservation methods for frozen foods, thermally processed foods and dehydrated foods are adequate in terms of costs, convenience, consumer acceptance and shelf-life. The most prominent promoter and developer of irradiated foods has been the US Quartermaster Corps. If in the future the marketing and consumer requirements change for different types of long-term shelf-life sterilised foods or for short-term non-refrigerated perishable foods, re-evaluation of present data or new research may successfully overcome the difficulties of consumer acceptance, high costs and possible toxicology of irradiated foods.

ANTI-BIOTICS

The original series of testing of anti-biotics as anti-microbial agents for food preservation in the 1950s included chlortetracycline, oxytetracycline, nisin, pimaricin and nystatin.[75] One of the general conclusions, based on the initial series of studies, is that anti-biotics exhibit selective anti-microbial activity; some are active against many gram-positive bacteria, some are active against gram-negative, and only a few show broad-spectrum activity.[75] One of the more important characteristics of anti-biotics is that they are not influenced by pH. The preservation action is static, and anti-biotic material must be continuously present.

In a typical product application to extend the shelf-life of fresh ground beef, Kohler *et al.*[76] used chlortetracycline in combination with either nystatin of myprozine. The results in Table 1 are based on storage tests of meat patties, tray packed and overwrapped with cellophane.

In another application, fresh fish was dipped for approximately 20 seconds in a water solution containing 10 ppm chlor- or oxytetracycline. A chelating agent such as EDTA was used to maintain the preservative action of the anti-biotic.[77]

TABLE 1
EFFECT OF ANTI-BIOTICS ON THE SHELF-LIFE OF MEAT PATTIES

Group	*Treatment before storage*	*Spoilage* (days)	*Discoloration* (days)
1	Control (untreated)	7	5
2	Chlortetracycline hydrochloride 3 ppm	11	13
3	Chlortetracycline hydrochloride 3 ppm plus 10 ppm nystatin	22+	20
4	Chlortetracycline hydrochloride 3 ppm plus 10 ppm myprozine	22+	22

It has been concluded that the use of anti-biotics, clinical or non-clinical, in situations where they can enter the food chain should be discouraged. Micro-organisms easily become resistant to anti-biotics and thus make it necessary to add more anti-biotic or use a different anti-biotic system.

Anti-biotic resistance, especially among the *Enterobactereaceae*, is plasmid mediated. Plasmids are extrachromosomal elements that behave like auxiliary chromosomes and have the ability to promote genetic transfer by conjugation. A plasmid is generally named for the particular property that is specifically its main characteristic. All plasmids are replicating genetic elements which direct their own replication and segregation during cellular division. These plasmids, also called resistance transfer factors (R-factors), have been found in most serotypes of *E. coli*, all four species of *Shigella*, and the Alkescens–Dispar group, many of the *Salmonella*, *Arizona*, *Citrobacter*, all species of *Proteus*, and members of the genera *Providencia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Aeromonas*, *Pseudomonas*, *Yersinia*, as well as others.[78] Plasmids can be transferred between strains of the same species, between species of the same genus, and between genera. In addition to anti-biotic resistance, other phenotypic characteristics are plasmid mediated. Biochemical characteristics, such as sugar fermentation, toxin formation, specific antigens, haemolysins and mucosal adhesion have

been associated with plasmids.[78,79] These factors, too, can be passed from one genus to another and thus make accurate identification—so important in food microbiology—virtually impossible.

Often, more than one character is transmitted at a time; multiple drug resistance is common.[80] Wachsmuth *et al.*[81] demonstrated that the plasmid mediation and transfer of heat-stable enterotoxin production and multiple drug resistance in enteropathogenic *E. coli* was responsible for a hospital outbreak. Concurrent transfer of both multiple drug resistance and enterotoxin production into *E. coli* K-12 was demonstrated also. The transfer of both drug resistance and toxin formation could occur between enteropathogenic *E. coli* and other coliforms (or even other genera) found in foods. Thus, because of the intergeneric transfer of plasmids and the possibility of multiple drug resistance, an *E. coli* resistant to anti-biotics present in foods could transfer such characters to a spoilage organism or a pathogen found in foods.

NEW FOOD ADDITIVES

Pharmaceutical and food companies have been active in the identification, development and testing for safety of new compounds that will preserve foods to avoid spoilage, illness and economic loss. Many of these compounds are described in the patent literature and some are listed in Table 2. Information on the food status of these compounds is not known by the authors. Enquiries on this subject should be directed to the companies involved.

TABLE 2
PARTIAL LIST OF CHEMICAL PRESERVATIVES IN PATENT LITERATURE[82]

Compound	*Food product*	*Company*	*US Patent*
Thioisomaltol	Fruits	General Foods	3 695 899 (1972)
Imidazoline	Beer	not assigned	3 440 056 (1969)
Lauric diethanol amide	Beer	not assigned	3 440 057 (1969)
Benzohydroxamic acid	General	Chas. Pfizer	3 446 630 (1969)
5-Aminohexahydro-pyrimidine	Fish	Warner-Lambert	2 963 374 (1960)
t-Butyl-hydroperoxide	Fish	Atlantic Richfield	3 622 351 (1971)
Phosphate peroxide	Fish	Takeda	3 545 982 (1970)
Chlorobromo dimethyl hydantoin	Dairy	Genhal	3 499 771 (1970)

OUTLOOK

The development and acceptance of new preservatives face a bleak future. Regulatory agencies will take a hard and forbidding look at new formulations. The prohibitive costs to test the safety of new compounds will discourage development by commercial concerns. Consequently, the emphasis in the future will probably be on new uses for old compounds and methods.

For example, lactic acid starter cultures have increased the shelf-life of liquid whole egg, mechanically deboned meats, and ground beef with only minor pH changes; therefore, the traditional purpose of starter cultures (production of large amounts of lactic acid quickly) does not appear to be operating here. Research is needed to clarify the mechanism by which lactic acid bacteria increase the shelf-life of meat products and the possible extension of the use of starters to other food systems.

Long-chain fatty acids and sequestrants are known bacterial inhibitors but are not used in foods to prevent bacterial growth. The bacterial membrane is sensitive to attack by fatty acids; this fact could be utilised to protect food systems from spoilage and pathogenic bacteria. Metal ions—especially iron—are needed for bacterial growth. Addition of purified chelating agents isolated from micro-organisms could play an important role in the extension of the shelf-life of foods. Addition of both a fatty acid and a sequestrant may provide a combination that spoilage and pathogenic bacteria could not overcome. Combinations containing sorbic acid and sodium acid pyrophosphate with or without nitrite were effective in preventing botulinal outgrowth.[83] Many combinations are possible with known food preservatives which will prevent bacterial growth effectively.

Controlled atmospheres coupled with a small decrease in water activity would provide conditions which would prevent the growth of many bacteria.

Radiation offers a preservative effect that can have minor effects on food quality and yet offer major protection against bacteria. If the human safety problem can be solved, radiation may well be the preservative of the future.

The food preservatives of the 1980s will probably not be new and unusual compounds but rather will be new and unusual combinations of old and tried compounds and methods.

REFERENCES

1. Bryan, F. L. (1976) *J. Milk Fd Technol.*, **39**, 289.
2. Oka, S. (1964) 'Mechanism of antimicrobial effect of various food preservatives', *Fourth International Symposium on Food Microbiology*, N. Molin (Ed.), Almqvist and Wiksell, Stockholm.
3. Johnston, M. A. and Loynes, R. (1971) *Can. Inst. Fd Technol. J.*, **4**, 179.
4. Ashworth, J. and Spencer, R. (1972) *J. Fd Technol.*, **7**, 111.
5. Tompkin, R. B., Christiansen, L. N. and Shaparis, A. B. (1978) *J. Fd Sci.*, **43**, 1368.
6. Tompkin, R. B., Christiansen, L. N. and Shaparis, A. B. (1978) *Appl. Environ. Microbiol.*, **35**, 59.
7. Tompkin, R. B., Christiansen, L. N. and Shaparis, A. B. (1978) *J. Fd Technol.*, **13**, 521.
8. Morris, S. G., Myers, J. S., Jr., Kip, M. L. and Riemenschneider, R. W. (1950) *J. Am. Oil Chem. Soc.*, **27**, 105.
9. Tompkin, R. B., Christiansen, L. N. and Shaparis, A. B. (1979) *Appl. Environ. Microbiol.*, **37**, 351.
10. Babel, F. J. (1977) *J. Dairy Sci.*, **60**, 815.
11. Speck, M. L. (1972) *J. Dairy Sci.*, **55**, 1019.
12. Reiter, B. (1978) *J. Dairy Res.*, **45**, 131.
13. Elliker, P. R., Sandine, W. E.. Hauser, B. A. and Moseley, W. K. (1964) *J. Dairy Sci.*, **47**. 680.
14. Task Force Report (October 1977) *The staphyloccal enterotoxin problem in fermented sausages*, Food Safety and Quality Service, US Department of Agriculture.
15. Daly, C., LaChance, M., Sandine, W. E. and Elliker, P. R. (1973) *J. Fd Sci.*, **38**, 426.
16. Niskanen, A. and Nurmi, E. (1976) *Appl. Environ. Microbiol.*, **31**, 11.
17. Christiansen, L. N., Tompkin, R. B., Shaparis, A. B., Johnston, R. W. and Dautter, D. A. (1975) *J. Fd Sci.*, **40**, 488.
18. Smith, J. L., Palumbo, S. A., Kissinger, J. C. and Huhtanen, C. N. (1975) *J. Milk Fd Technol.*, **38**, 150.
19. Smith, J. L., Huhtanen, C. N., Kissinger, J. C. and Palumbo, S. A. (1975) *Appl. Microbiol.*, **30**, 759.
20. Raccach, M. and Baker, R. C. (1979) *J. Fd Sci.*, **44**, 90.
21. Raccach, M. and Baker, R. C. (1978) *J. Fd Prot.*, **41**, 703.
22. Bryan, F. L. (1976) 'Staphyloccus aureus', *Food microbiology: public health and spoilage aspects*, M. P. Defigueiredo and D. F. Splittstoesser (Eds.), Avi Publishing Co., Westport, Conn., USA, pp. 13–128.
23. Daly, C., Sandine, W. E. and Elliker, P. R. (1972) *J. Milk Fd Technol.*, **35**, 349.
24. Bartholomew, D. T. and Blumer, T. N. (1977) *J. Fd Sci.*, **42**, 498.
25. Reddy, S. G., Henrickson, R. L. and Olson, H. C. (1970) *J. Fd Sci.*, **35**, 787.
26. Shelef, L. A. (1977) *J. Fd Sci.*, **42**, 1172.
27. Riemann, H., Lee, W. H. and Genigeorgis, C. (1972) *J. Milk Fd Technol.*, **35**, 514.
28. Gilliland, S. E. and Speck, M. L. (1977) *J. Fd Prot.*, **40**, 820.
29. Branen, A. L., Go, H. C. and Genske, R. P. (1975) *J. Fd Sci.*, **40**, 446.
30. Pulusani, S. F., Rao, D. R. and Sunki, G. R. (1979) *J. Fd Sci.*, **44**, 575.
31. Nieman, C. (1954) *Bacteriol. Rev.*, **18**, 147.
32. Hamilton, W. A. (1968) *J. Gen. Microbiol.*, **50**, 441.
33. Hart, P. D'Arcy, Lovelock, J. E. and Nash, J. (1962) *J. Hyg. Camb.*, **60**, 509.
34. Kondo, E. and Kanai, K. (1972) *Japan J. Med. Sci. Biol.*, **25**, 1.
35. Kondo, E. and Kanai, K. (1976) *Japan J. Med. Sci. Biol.*, **29**, 25.
36. Galbraith, H., Miller, T. B., Paton, A. M. and Thompson, J. K. (1971) *J. Appl. Bacteriol.*, **34**, 803.
37. Kabara, J. J., Swieczkowski, D. M., Conley, A. J. and Truent, J. P. (1972) *Antimicrob. Agents Chemother.*, **2**, 23.
38. Kabara, J. J., Vrable, R. and Lie Ken Jie, M. S. F., (1977) *Lipids*, **12**, 753.
39. Sheu, C. W. and Freese, E. (1972) *J. Bacteriol.*, **111**, 516.
40. Freese, E., Sheu, C. W. and Galliers, E. (1973) *Nature*, **241**, 321.
41. Galbraith, H. and Miller, T. B. (1973) *J. Appl. Bacteriol.*, **36**, 635.
42. Galbraith, H. and Miller, T. B. (1973) *J. Appl. Bacteriol.*, **36**, 647.
43. Galbraith, H. and Miller, T. B. (1973) *J. Appl. Bacteriol.*, **36**, 659.
44. Sheu, C. W. and Freese, E. (1973) *J. Bacteriol.*, **115**, 869.
45. Fay, J. P. and Farias, R. N. (1977) *J. Bacteriol.*, **132**, 790.
46. Kato, A. and Arima, K. (1971) *Biochem. Biophys. Res. Commun.*, **42**, 596.
47. Kabara, J. J. (1975) *J. Cosmet. Perfum.*, **90**, 21.
48. Chang, H. C. and Branen, A. L. (1975) *J. Fd Sci.*, **40**, 349.
49. Fung, D. Y., Taylor, S. and Kahan, J. (1977) *J. Fd Safety*, **1**, 39.
50. Robach, M. C., Smoot, L. A. and Pierson, M. D. (1977) *J. Fd Prot.*, **40**, 549.
51. Klindworth, K. J., Davidson, P. M., Brekke, C. J. and Branen, A. L. (1979) *J. Fd Sci.*, **44**, 564.
52. Furia, T. E. (1973) 'Sequesterants in foods', *CRC Handbook of Food Additives*, 2nd edn, T. E. Furia (Ed.), Chemical Rubber Co. Press Inc., Cleveland, Ohio.
53. Schade, A. L. and Caroline, L. (1944) *Science*, **100**, 14.
54. Moats, W. A. (1978) *J. Fd Prot.*, **41**, 919.
55. Law, B. A. and Reiter, B. (1977) *J. Dairy Res.*, **44**, 595.
56. Byers, R. B. and Arceneaux, J. E. L. (1977) 'Microbial transport and utilization of iron', *Microorganisms and minerals*, E. D. Weinberg (Ed.), Marcel Dekker Inc., New York, pp. 215–49.
57. Weinberg, E. D. (1978) *Microbiol. Rev.*, **42**, 45.
58. Wawszkiewicz, E. J., Schneider, H. A., Starcher, B., Pollack, J. and Neilands, J. B. (1971) *Proc. Natl. Acad. Sci., USA*, **68**, 2870.
59. Cummins, A. S., Daun, H., Gilbert, S. G. and Henig, Y. (1974) US Patent 3 795 749.
60. Benedict, R. C., Strange, E. D., Palumbo, S. and Swift, C. E. (1975) *J. Agric. Fd Chem.*, **23**, 1208.
61. Huffman, D. L., Davis, K. A., Marple, D. N. and McGuire, J. A. (1975) *J. Fd Sci.*, **40**, 1229.
62. Mermelstein, N. H. (1979) *Fd Technol.*, **33**(7), 32.
63. Fiddler, W., Doerr, R. C. and Wasserman, A. E. (1970) *Agric. Fd Chem.*, **18**, 310.
64. Fiddler, W., Wasserman, A. E. and Doerr, R. C. (1970) *Agric. Fd Chem.*, **18**, 934.

65. HANDFORD, P. M. and GIBBS, B. M. (1964) 'Antibacterial effects of smoke constituents on bacteria isolated from bacon', *Microbial inhibitors in food*, N. Molin (Ed.), Almqvist and Wiksell, Stockholm, pp. 333–46.
66. HOUBEN, J. H. (1974) *Voedingsmiddelentechnologie*, **7**(41), 8.
67. HOUBEN, J. H. (1975) *Voedingsmiddelentechnologie*, **8**(15), 5.
68. POWERS, J. J. (1964) 'Action of anthocyanin and related compounds on bacterial cells', *Microbial inhibitors in food*, N. Molin (Ed.) Almqvist and Wiksell, Stockholm.
69. JURD, L. K., JR, MIHARA, K. and STANLEY, W. L. (1971) *Appl. Microbiol.*, **21**, 507.
70. SMALE, H., WILSON, R. A. and KEIL, H. L. (1964) *J. Phytopathol.*, **64**, 748.
71. BLOOMFIELD, S. (1974) *J. Appl. Bacteriol.*, **37**, 117.
72. URBAIN, W. M. (1978) *Adv. Fd Res.*, **24**, 155.
73. International Atomic Energy Agency (1978) *Food preservation by irradiation*, (*IAEA Proc. Ser.*), Vol. 1 and 2, Inst. Atom. Sci. Agr., Wageningen, Netherlands, 21–25 Nov, 1977 (Sponsors: Food & Agr. Org./WHO), IAEA, Vienna.
74. WIERBICKI, E., HEILIGMAN, F. and WASSERMAN, A. E. (1976) 'Irradiation as a conceivable way of reducing nitrites and nitrates in cured meats', *Proc. 2nd Int. Symp. Nitrite Meat Pros.*, Zeist, 1976, Pudoc, Wageningen, Netherlands, pp. 75–81.
75. CHICHESTER, D. F. and TANNER, F. W. (1973) 'Antimicrobial food additives'. In: *Handbook of food additives*, 2nd edn, T. E. Furia (Ed.) Chemical Rubber Co. Inc., Cleveland, Ohio, USA.
76. KOHLER, A. R., MILLER, W. H. and WINDLAN, H. M., American Cynamid Co. (1962) US Patent 3 050 401.
77. FURIA, T. E., Geigy Chemical Corp. (1971) US Patent 3 563 770.
78. HOWELL, C. and MARTIN, W. J. (1978) *J. Fd Prot.*, **41**, 44.
79. TWEDT, R. M. and BOUTIN, B. K. (1979) *J. Fd Prot.*, **42**, 161.
80. DAVIES, J. and SMITH, D. I. (1978) *Ann. Rev. Microbiol.*, **32**, 469.
81. WACHSMUTH, K. K., FALKOW, S. and RYDER, R. W. (1976) *Infect. Immun.*, **14**, 403.
82. PINTAURO, N. D. (1974) *Food additives to extend shelf life* (*Food Technology Review No. 17*), Noyes Data Corp., Park Ridge, N. J., USA.
83. IVEY, F. J. and ROBACH, M. C. (1978) *J. Fd Sci.*, **43**, 1782.